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Evaluation of the GenoType MTBDRplus Assay for Rifampin and Isoniazid Susceptibility Testing of *Mycobacterium tuberculosis* Strains and Clinical Specimens[▽]

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The new GenoType MTBDRplus assay (Hain Lifescience GmbH, Nehren, Germany) was tested with 125 clinical isolates and directly with 72 smear-positive sputum specimens for its ability to detect rifampin (RMP) and isoniazid (INH) resistance in *Mycobacterium tuberculosis* complex (MTBC) strains. In total, 106 RMP^r/INH^r, 10 RMP^s/INH^r, and 80 RMP^s/INH^s MTBC strains were comparatively analyzed with the new and the old MTBDR assays. Besides the detection of mutations within the 81-bp hot spot region of *rpoB* and *katG* codon 315, the GenoType MTBDRplus assay is designed to detect mutations in the regulatory region of *inhA*. The applicability of the new assay directly to specimens was shown, since 71 of 72 results for smear-positive sputa and all 125 results for clinical isolates were interpretable and no discrepancies compared with the results of real-time PCR or DNA sequencing were obtained. In comparison to conventional drug susceptibility testing, both assays were able to identify RMP resistance correctly in 74 of 75 strains (98.7%) and 30 of 31 specimens (96.8%). The misidentification of RMP resistance was obtained for two strains containing *rpoB* P533L mutations. Compared to the old MTBDR assay, the new GenoType MTBDRplus assay enhanced the rate of detection of INH resistance from 66 (88.0%) to 69 (92.0%) among the 75 INH-resistant strains and 36 (87.8%) to 37 (90.2%) among the 41 specimens containing INH-resistant strains. Thus, the new GenoType MTBDRplus assay represents a reliable and upgraded tool for the detection of INH and RMP resistance in strains or directly from smear-positive specimens.

The worldwide increase in the rates of multidrug-resistant (MDR) tuberculosis (resistance to at least rifampin [RMP] and isoniazid [INH]) has made the timely identification of resistant *Mycobacterium tuberculosis* complex (MTBC) strains to achieve effective disease management and to prevent their spread extremely important. INH and RMP are the most important first-line antituberculosis drugs, and resistance to these drugs often results in treatment failures and fatal clinical outcomes (6, 7).

Recently, nonradiometric fully automated systems that are used to screen for resistance and that have technical and safety advantages have been introduced (23). However, the time for resistance testing still is about 7 to 10 days, beginning from the time that a positive culture is obtained (23). The most rapid results could be achieved by direct testing of patient specimens by fast molecular methods (11, 25). These methods are based on the knowledge that resistance to RMP and INH in *M. tuberculosis* is most often attributed to mutations in the *rpoB*, *katG*, and *inhA* genes. By targeting mutations in the 81-bp "core region" of the *rpoB* gene, more than 95% of all RMP-resistant strains can be detected (28). On the contrary, the mutations that cause INH resistance are located in several genes and regions. Between 50% and 95% of INH-resistant strains have been found to contain mutations in codon 315 of the *katG* gene (18, 20, 28), between 20 and 35% of INH-resistant strains have been found to contain mutations in the *inhA* regulatory region (20, 22, 28), and an additional 10 to 15% of INH-resistant strains had mutations in the *ahpC-oxyR* intergenic region (13, 22, 28), often in conjunction with *katG* mutations outside of codon 315 (26). In a recent study, the strong statistical association between specific mutations in the *katG*, *inhA*, and *ahpC* genes and INH resistance could be confirmed (8). The authors estimated that a simple test for five molecular markers is able to detect 74% of INH-resistant (INH^r) isolates; 0 to 5% of the INH^r *M. tuberculosis* isolates had mutations in the *inhA* open reading frame and 8 to 20% had mutations in the *inhA* promoter region (8, 20, 31).

DNA strip assays targeting *rpoB* (INNO-LiPA Rif; Innogenetics N.V., Ghent, Belgium) or *rpoB* plus *katG* (GenoType MTBDR; Hain Lifescience GmbH, Nehren, Germany) were developed and evaluated for use with *M. tuberculosis* cultures and smear-positive specimens (1, 11, 16, 17, 29). The DNA strip assays are based on a multiplex PCR in combination with reverse hybridization. Either the omission of a wild-type band or the appearance of bands of DNA signals representing specific mutations indicates the existence of a resistant strain.

In order to enlarge the capacity for the detection of drug resistance, the new GenoType MTBDRplus assay was developed. The assay has the ability to detect a broader variety of *rpoB* gene mutations and *inhA* gene mutations. By covering mutations in the regulatory region of *inhA*, it can be expected that additional INH-resistant strains can be detected.

The aim of the present study was to determine the sensitivity and accuracy of the new MTBDRplus assay in comparison to those of the MTBDR assay for the detection of INH and RMP resistance-associated mutations in *rpoB*, *katG*, and *inhA* from

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TABLE 1. GenoType MTBDRplus test results in comparison with GenoType MTBDR test results for detection of RMP and INH resistance in 75 MDR strains[a]

No. (%) of strains	Sequencing data, nucleotide/amino acid change(s)[b]	MTBDR assay			MTBDRplus assay			
		RMP pattern (*rpoB*)	INH pattern (*katG*)	Result	RMP pattern (*rpoB*)	INH pattern (*katG*)	INH pattern (*inhA*)	Result
45 (60.0)	*rpoB* S531L, *katG* 315T1[c]	ΔWT 5, Mut 3	ΔWT, Mut T1	MDR	ΔWT 8, Mut 3	ΔWT, Mut T1	WT	MDR
2 (2.7)	*rpoB* S531L, *katG* S315T1, *inhA* T8C	ΔWT 5, Mut 3	ΔWT, Mut T1	MDR	ΔWT 8, Mut 3	ΔWT, Mut T1	ΔWT1, Mut T8C	MDR
1 (1.3)	*rpoB* S531L, *katG* S315T1, *inhA* C15T	ΔWT 5, Mut 3	ΔWT, Mut T1	MDR	ΔWT 8, Mut 3	ΔWT, Mut T1	ΔWT1, Mut C15T	MDR
2 (2.7)	*rpoB* S531L, *inhA* C15T	ΔWT 5, Mut 3	WT	RMP^r	ΔWT 8, Mut 3	WT	ΔWT1, Mut C15T	MDR
2 (2.7)	*rpoB* S531L	ΔWT 5, Mut 3	WT	RMP^r	ΔWT 8, Mut 3	WT	WT	RMP^r
2 (2.7)	*rpoB* S531L, *katG* S315N	ΔWT 5, Mut 3	ΔWT	MDR	ΔWT 8, Mut 3	ΔWT	WT	MDR
1 (1.3)	*rpoB* S531W, *katG* 315T1	ΔWT 5	ΔWT, Mut T1	MDR	ΔWT 8	ΔWT, Mut T1	WT	MDR
1 (1.3)	*rpoB* S531W, *inhA* C15T	ΔWT 5	WT	RMP^r	ΔWT 8	WT	ΔWT1, Mut C15T	MDR
1 (1.3)	*rpoB* S531W	ΔWT 5	WT	RMP^r	ΔWT 8	WT	WT	RMP^r
1 (1.3)	*rpoB* S531P, *katG* S315T1	ΔWT 5	ΔWT, Mut T1	MDR	ΔWT 8	ΔWT, Mut T1	WT	MDR
1 (1.3)	*rpoB* S531L, *katG* S315T1, T2	ΔWT 5, Mut 3	ΔWT, Mut T1, T2	MDR	ΔWT 8, Mut 3	ΔWT, Mut T1, Mut T2	WT	MDR
1 (1.3)	*rpoB* H526D, *katG* 315T1	ΔWT 4, Mut 2B	ΔWT, Mut T1	MDR	ΔWT 7, Mut 2B	ΔWT, Mut T1	WT	MDR
2 (2.7)	*rpoB* H526L, *katG* 315T1	ΔWT 4	ΔWT, Mut T1	MDR	ΔWT 7	ΔWT, Mut T1	WT	MDR
2 (2.7)	*rpoB* H526N, *katG* 315T1	ΔWT 4	ΔWT, Mut T1	MDR	ΔWT 7	ΔWT, Mut T1	WT	MDR
1 (1.3)	*rpoB* H526N, *katG* S315T1, *inhA* C15T	ΔWT 4	ΔWT, Mut T1	MDR	ΔWT 7	ΔWT, Mut T1	ΔWT1, Mut C15T	MDR
2 (2.7)	*rpoB* H526R, *katG* S315T1, *inhA* C15T	ΔWT 4	ΔWT, Mut T1	MDR	ΔWT 7	ΔWT, Mut T1	ΔWT1, Mut C15T	MDR
1 (1.3)	*rpoB* H526R, *ahpC* C52T	ΔWT 4	WT	RMP^r	ΔWT 7	WT	WT	RMP^r
1 (1.3)	*rpoB* D516V, *katG* 315T1	ΔWT 2, Mut 1	ΔWT, Mut T1	MDR	ΔWT 3, 4, Mut 1	ΔWT, Mut T1	WT	MDR
1 (1.3)	*rpoB* D516V, *katG* S315T1, *inhA* C15T	ΔWT 2, Mut 1	ΔWT, Mut T1	MDR	ΔWT 3, 4, Mut 1	ΔWT, Mut T1	ΔWT1, Mut C15T	MDR
1 (1.3)	*rpoB* N518I, *katG* S315T1	ΔWT 2, Mut 1	ΔWT, Mut T1	MDR	ΔWT 3, 4, Mut 1	ΔWT, Mut T1	WT	MDR
1 (1.3)	*rpoB* del514-516, *katG* S315T1	ΔWT 1, 2	ΔWT, Mut T1	MDR	ΔWT 2–4	ΔWT, Mut T1	WT	MDR
1 (1.3)	*rpoB* S522Q, *katG* 315T1	ΔWT 3	ΔWT, Mut T1	MDR	ΔWT 5, 6	ΔWT, Mut T1	WT	MDR
1 (1.3)	*rpoB* Q513P	ΔWT 1	WT	RMP^r	ΔWT 2, 3	WT	WT	RMP^r
1 (1.3)	*rpoB* V176F, *katG* K152T, *ahpC* G48A	WT	WT	Susc.	WT	WT	WT	Susc.

[a] Resistance was determined by conventional DST. ^{r}, resistant; Susc., susceptible; Δ deletion; WT, wild type; Mut, mutation.
[b] According to previously published data (28); the GenBank accession numbers are L27989 for the *rpoB* gene, X68081 for the *katG* gene, U66801 for the *inhA* gene, and U16243 for the *ahpC-oxyR* intergenic region.
[c] S315T1, AGC→ACC/Ser→Thr exchange; S315T2, AGC→ACA/Ser→Thr.

culture specimens and directly from smear-positive clinical specimens.

MATERIALS AND METHODS

Culture strains. A set of 75 previously characterized MDR strains obtained from patients living in Germany in 2001 was analyzed (10). The DNA preparation method is described elsewhere (10). As controls, 50 randomly chosen and previously characterized fully susceptible MTBC strains were used.

Clinical specimens. Sputum specimens sent to the National Reference Laboratory (from 2005 to 2006) were processed by the conventional *N*-acetyl-L-cysteine–NaOH method (final NaOH concentration, 1%) (5). After decontamination, the concentrated sediment was suspended in 1.0 to 1.5 ml sterile phosphate buffer (pH 7.0), and smears were prepared by the Ziehl-Neelsen staining method (14). After inoculation of solid and liquid media for growth detection, the leftover sediment of the decontaminated sputum specimen was stored at −20°C. After growth of the cultures, species identification and, in cases in which MTBC strains were identified, drug susceptibility testing (DST) were performed. The DST results were used to enable the selection of a representative collection of 72 smear-positive specimens. The leftover sediment of these selected specimens was thawed and used for testing by the MTBDR and MTBDRplus assays. For this, 500 μl of each sample was centrifuged at 13,000 × *g* for 15 min, the supernatant was discarded, and the pellet was resuspended in 100 μl distilled water. Subsequently, the suspension was boiled for 20 min and incubated in a sonic water bath at room temperature for 15 min.

Identification of MTBC strains from clinical specimens. For the identification and differentiation of MTBC strains from the grown cultures, the GenoType MTBC assay was performed according to the instructions of the manufacturer (Hain Lifescience GmbH).

DST. DST with INH and RMP was performed by the BACTEC MGIT 960 method (MGIT 960; Becton Dickinson Diagnostic Systems, Sparks, MD) and the proportion method on Löwenstein-Jensen medium (LJ) (3). Tests were performed with the standard critical concentrations of INH (0.1 μg/ml for the MGIT 960 method and 0.25 μg/ml for LJ) and RMP (1 μg/ml for the MGIT 960 method and 40 μg/ml for LJ).

Genotypic characterization. All 125 strains derived from cultures were analyzed by real-time PCR and/or DNA sequencing of the key regions involved in the development of resistance (*rpoB*, *katG inhA*, and *ahpC*) (12, 24). Primers inhA 3F and inhA 4R (19), whose sequences flank the region encoding amino acid Ser94 of *inhA*, were used to amplify a 517-bp *inhA* gene fragment. Primers katG 290F (5′-ACT ACG GGC CGC TGT TTA TC-3′) and katG 583R (5′-T CCTTGCCCCAATAGACCTC-3′), which were designed in this study, were used to amplify a 250-bp fragment of *katG* which included a *katG* region (codons 108 to 138) with INH resistance-associated mutations (8). Direct sequencing of the PCR products was carried out with an ABI Prism 3100 capillary sequencer (Applied Biosystems) and the ABI Prism BigDye Terminator kit (version 1.1), according to the manufacturer's instructions. For the 72 smear-positive specimens, sequencing of specific DNA fragments was performed only if any discrepancy was observed between conventional DST and the MTBDR assays. DNA sequencing analysis was also performed if a mutation was detected only by omission of a wild-type band in one of the assays.

GenoType MTBDR assays. Since for all samples the results of DST were known, both the MTBDR assay and the new MTBDRplus assay were performed in a blinded manner.

For all strains derived from cultures, the strip assays were performed as recommended by the manufacturer. Briefly, for amplification, 35 μl of a primer-nucleotide mixture (provided with the kit), amplification buffer containing 2.5 mM $MgCl_2$, 1.25 U hot start *Taq* polymerase (QIAGEN, Hilden, Germany), and

5 μl of a preparation of chromosomal DNA in a final volume of 50 μl were used. The amplification protocol consisted of 15 min of denaturation at 95°C, followed by 10 cycles comprising 30 s at 95°C and 120 s at 58°C; an additional 20 cycles comprising 25 s at 95°C, 40 s at 53°C, and 40 s at 70°C; and a final extension at 70°C for 8 min. Hybridization and detection were performed in an automated washing and shaking device (Profiblot; Tekan, Maennedorf, Switzerland). The hybridization procedure was performed at 45°C for 0.5 h, followed by washing steps and the colorimetric detection of the hybridized amplicons. After a final wash, the strips were air dried and fixed on paper.

For the sputum specimens, an altered amplification protocol was applied which consisted of 15 min of denaturation at 95°C, followed by 10 cycles comprising 30 s at 95°C and 120 s at 58°C; an additional 35 cycles comprising 25 s at 95°C, 40 s at 53°C, and 40 s at 70°C; and a final extension at 70°C for 8 min. Hybridization and detection were performed as described above.

The MTBDR strip contains 17 probes, including 5 amplification and hybridization controls to verify the test procedures. For the detection of RMP resistance, five *rpoB* wild-type probes (probes WT1 to WT5) encompass the region of the *rpoB* gene encoding amino acids 509 to 534. Four probes (probes *rpoB* MUT D516V, *rpoB* MUT H526Y, *rpoB* MUT H526D, and *rpoB* MUT S531L) specifically target the most common mutations. For the detection of INH resistance, one probe covers the wild-type S315 region of *katG*, while two others (probes *katG* MUT T1 and MUT T2) are designed to assess the AGC-to-ACC (S315T) and the AGC-to-ACA (S315T) mutations.

The new MTBDRplus strip contains, in addition to all probes included in the MTBDR assay, three further *rpoB* wild-type probes to fill the gaps between the probes of the old MTBDR assay and to increase the detectable *rpoB* DNA fragment to amino acids 505 to 533. Furthermore, the promoter region of the *inhA* gene is included on the new strip and encompasses the regions from positions −9 to −22 for the *inhA* WT1 probe and positions −1 to −12 for the *inhA* WT2 probe. Four mutations (−15C/T, −16A/G, −8T/C, and −8T/A) can be targeted with the *inhA* MUT1, MUT2, MUT3A, and MUT3B probes. Again, either the omission of a wild-type probe or the staining of a mutant probe is an indication of a resistant strain.

RESULTS

A total of 117 drug-resistant isolates (75 from cultures and 42 from smear-positive specimens) and 80 pansusceptible isolates (50 from cultures and 30 from smear-positive specimens) characterized previously were included in the study and tested by both assays. Overall, interpretable results were obtained for 196 isolates. The *rpoB*-specific bands were repeatedly weak for only one smear-positive specimen and therefore were excluded from the analysis. In general, interpretation of the results for both test strips was easy, but the intensities of the different hybridization bands varied. The TUB control band that should be positive in all MTBDR tests for MTBC strains was, in some cases, very weak with smear-positive specimens in the MTBDR assay but had good visibility in the new MTBDRplus assay.

Results of the GenoType MTBDR and MTBDRplus assays with DNA of MDR and pansusceptible culture isolates. A total of 125 strains (75 MDR strains and 50 pansusceptible strains precharacterized by conventional DST; real-time PCR; and/or sequencing of the *rpoB*, *katG*, *inhA*, and *ahpC* genes) were tested by both assays. Both MTBDR assays accurately detected MTBC isolates by detection of the specific band in all (100%) strains. The interpretation of the banding pattern was comparable between the assays. Moreover, no discrepancies between the two assays and the real-time PCR/DNA sequencing results could be detected.

All 50 pansusceptible strains showed wild-type patterns by both assays. For 65 (86.7%) of the 75 MDR strains, the results of both assays were in agreement with the results of both DST and real-time PCR/DNA sequencing (Table 1). Both assays indicated RMP resistance in 74 strains (98.7%); only 1 strain could not be recognized as RMP resistant by the assays since it had a mutation outside the *rpoB* hot spot region. Both assays correctly identified the INH resistance of 65 strains (86.7%), comprised of 63 strains with a *katG* S315T mutation and 2 strains with an S315N mutation that was detected by the omission of the wild-type band. For one of these strains, both assays indicated the presence of two mutations (*katG* Mut T1 and *katG* Mut T2). DNA sequencing confirmed both mutations in this strain. Seven of the 63 strains with S315T mutations in *katG* gene had additional *inhA* mutations (5 strains with C15T alterations and 2 strains with T8C alterations).

The MTBDR assay indicated INH susceptibility in three

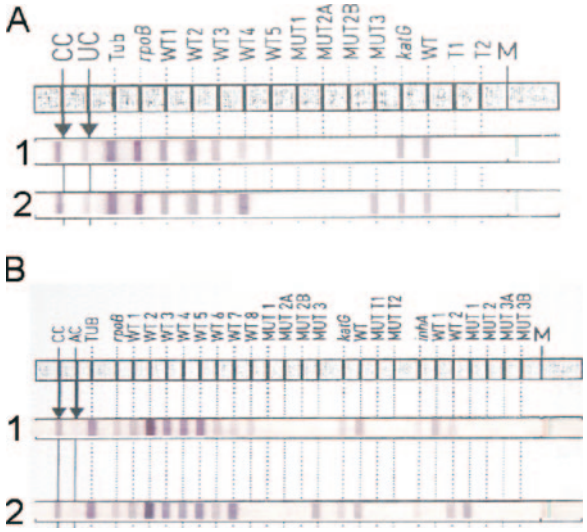

FIG. 1. Representative patterns of a pansusceptible strain (rows 1) and an MDR strain (rows 2) which had an *inhA* C15T mutation in the regulatory region of *inhA* obtained by the new MTBDRplus assay (B) but not the MTBDR assay (A). The positions of the oligonucleotides and the marker lines are given. The targeted genes and specificity are shown from left to right, as follows: for the MTBDR assay (A), conjugate control (CC); amplification control (UC); *M. tuberculosis* complex-specific control (Tub); *rpoB* amplification control; *rpoB* wild-type probes WT1 to WT5 located in the hot spot region WT 1 to WT 5, respectively; *rpoB* mutant probes (probes MUT1, MUT2A, MUT2B, and MUT3) with mutations in codons *rpoB* D516V, H526Y, H526D, and S531L, respectively; *katG* amplification control; *katG* codon 315 wild-type probe (WT); and *katG* codon 315 mutation probes (T1 and T2, respectively) with AGC-to-ACC (S315T1) and AGC-to-ACA (S315T2) exchanges, respectively. The MTBDR plus assay (A) has three additional *rpoB* wild-type probes (resulting in probes WT1 to WT8) and targets the regulatory region of the *inhA* gene with the *inhA* amplification control (WT 1 to WT 8, respectively); *inhA* gene wild-type probes WT1, which spans the region from positions −9 to −22, and WT, which spans the region from positions −1 to −12; and *inhA* mutant probes MUT1, MUT2, MUT3A, and MUT3B with mutations −15C/T, −16A/G, −8T/C, and −8T/A, respectively (MUT 1, MUT 2A, MUT 2B, and MUT 3, respectively). Pansusceptible isolate 1 was positive with the wild-type *rpoB* and *katG* probes of the MTBDR assay and, additionally, with the *inhA* wild-type probes of the MTBDRplus assay. MDR isolate 2 showed RMP resistance in both assays (for the MTBDR assay, the omission of *rpoB* WT 5 and positivity for *rpoB* Mut 3; for the MTBDRplus assay, the omission of *rpoB* WT 8 and positivity for *rpoB* Mut 3). Concerning INH resistance, isolate 2 had to be interpreted as INH susceptible (*katG* wild-type probe) by the MTBDR assay, whereas the MTBDRplus assay indicated INH resistance (omission of the *inhA* WT1 probe and positivity for the *inhA* MUT1 probe). Lanes M, colored markers.

TABLE 2. GenoType MTBDRplus test results in comparison with GenoType MTBDR test results for detection of MDR, INH-resistant, and fully susceptible strains in smear-positive sputum specimens

Specimen[a] (*n*)	No. (%) of strains	Sequencing data, nucleotide/amino acid change(s)	MTBDR assay[b]			MTBDRplus assay[b]			
			RMP pattern (*rpoB*)	INH pattern (*katG*)	Result[c]	RMP pattern (*rpoB*)	INH pattern (*katG*)	INH pattern (*inhA*)	Result
MDR (31)	19 (61.3)	Not done	ΔWT 5, Mut 3	ΔWT, Mut T1	MDR	ΔWT 8, Mut 3	ΔWT, Mut T1	WT	MDR
	1 (3.2)	Not done	ΔWT 5, Mut 3	ΔWT, Mut T1	MDR	ΔWT 8, Mut 3	ΔWT, Mut T1	ΔWT 1, Mut C15T	MDR
	1 (3.2)	*rpoB* S531L, *katG* WT, *inhA* WT, *ahpC* WT	ΔWT 5, Mut 3	WT	RMP^r	ΔWT 8, Mut 3	WT	WT	RMP^r
	1 (3.2)	*rpoB* S531L, *katG* S315N	ΔWT 5, Mut 3	ΔWT	MDR	ΔWT 8, Mut 3	ΔWT	WT	MDR
	1 (3.2)	*rpoB* S531L/WT mix, *katG* S315T/WT Mix	WT (5), Mut 3	WT, Mut T1	MDR	WT (8), Mut 3	WT, Mut T1	WT	MDR
	1 (3.2)	*rpoB* WT, *katG* S315N	WT	ΔWT	INH^r	WT	ΔWT	WT	INH^r
	3 (9.7)	*rpoB* H526D, *katG* S315T	ΔWT 4, Mut 2b	ΔWT, Mut T1	MDR	ΔWT 7, Mut 2b	ΔWT, Mut T1	WT	MDR
	3 (9.7)	*rpoB* H526D, *katG* S315T	ΔWT 4, Mut 2B	ΔWT, Mut T1	MDR	ΔWT 7, Mut 2B	ΔWT, Mut T1	ΔWT 1, Mut C15T	MDR
	1 (3.2)	*rpoB* H526R/WT mix, *katG* S315N/WT mix	WT (4)	(WT), Mut T1	MDR	WT (7)	(WT), Mut T1	WT	MDR
INH^r (10)	2 (20)	Not done	WT	ΔWT, Mut T1	INH^r	WT	ΔWT, Mut T1	WT	INH^r
	1 (10)	Not done	WT	ΔWT, Mut T1	INH^r	WT	ΔWT, Mut T1	ΔWT 1, Mut C15T	INH^r
	3 (30)	*katG* WT, *inhA* WT, *ahpC* WT	WT	WT	Susc.	WT	WT	WT	Susc.
	1 (10)	*inhA* C15T	WT	WT	Susc.	WT	WT	ΔWT 1, Mut C15T	INH^r
	1 (10)	*rpoB* L533P, *katG* S315T	ΔWT 5	ΔWT, Mut T1	MDR	ΔWT 8	ΔWT, Mut T1	WT	MDR
	1 (10)	*rpoB* L533P, *katG* S315N	ΔWT 5	ΔWT	MDR	ΔWT 8	ΔWT	WT	MDR
	1 (10)	*rpoB* WT[d]	WT (4, 5)	ΔWT, Mut T1	INH^r	WT (7, 8)	ΔWT, Mut T1	ΔWT 1, Mut C15T	INH^r
Susceptible (30)	30 (100)	Not done	WT	WT	Susc.	WT	WT	WT	Susc.

[a] Resistance was determined by conventional DST.
[b] WT, wild-type pattern with all respective bands visible; Δ, missing bands; weak or very weak bands are listed in parentheses. Mut, mutation.
[c] r, resistant; Susc., susceptible.
[d] This isolate contained a second *rpoB* sequence derived from a mycobacterial strain not further characterized.

strains (4.0%), whereas the MTBDRplus assays showed INH resistance due to a C15T alteration in *inhA* (Fig. 1). Both MTBDR assays failed to detect INH resistance in seven strains (9.3%) (two of these had *ahpC* mutations).

Provided that conventional DST is the "gold standard," the sensitivities of the MTBDR assay were 98.7% for RMP resistance detection and 88.0% for INH resistance detection. While the sensitivity of the MTBDRplus assay was identical to that of the MTBDR assay for the detection of RMP resistance, it was slightly higher than that of the MTBDR assay for the detection of INH resistance (92.0%). Both assays had specificities of 100% for the detection of INH resistance.

Results of GenoType MTBDR and MTBDRplus assays with smear-positive sputum specimens with strains resistant to RMP and INH or INH but not RMP and with pansusceptible strains. A total of 72 smear-positive specimens were chosen for analysis, including 32 MDR strains assessed with the MGIT 960 system. Ten strains were resistant to INH but not RMP, and 30 strains were pansusceptible (Table 2). An interpretable result could be achieved for 71 of the 72 specimens. The sputum positivity of the samples ranged from 1+ to 4+, according to the German guidelines (5), but no correlation between the intensities of the banding patterns and the amount of acid-fast bacteria could be observed. Thus, each of the 71 specimens contained sufficient extractable DNA without any inhibitors to yield this high rate of recovery efficiency.

For 29 (93.5%) of the 31 MDR strains, both MTBDR assay results were in agreement with the results of conventional DST and DNA sequencing (Table 2). One (3.2%) MDR strain had the RMP wild-type banding pattern by both assays. However, DNA sequencing was also unable to find any mutations in the *rpoB* hot spot region and an *rpoB* region further upstream. For another MDR strain (3.2%), the MTBDR assays failed to indicate the INH resistance of the strain. With additional DNA sequencing of the regulatory region of *ahpC* and parts of the *katG* and *inhA* open reading frame regions, no mutation indicating INH resistance was detected. In two cases the MTBDR assays showed a mixture of banding patterns indicating both resistance and susceptibility to RMP and INH (weak *rpoB* wild-type result with *rpoB*-specific probe MUT2 and three mutation-specific bands for one strain and a weak *katG* wild-type result with strong *katG* mutation-specific bands with probe MUT T1 for the other; Table 2). DNA sequencing confirmed the prevalence of both the wild-type and the mutated sequences.

For the group of INH^r RMP^s strains, the rate of detection of INH resistance was lower compared to that for the MDR strains. For 4 of the 10 specimens, the old MTBDR test indi-

cated INH susceptibility (no detectable mutation at *katG* codon 315). However, the new MTBDRplus assay detected INH resistance due to a C15T exchange in *inhA* in one of these four strains. Furthermore, in two of the INH^r RMP^s strains, mutations were indicated by the omission of MTBDR and MTBDRplus *rpoB* bands 5 and 8, respectively. DNA sequencing confirmed this alteration as an CTG-to-CCG exchange in *rpoB* codon 533.

Sensitivities and specificities of the GenoType MTBDR and MTBDRplus assays. Overall, both assays had identical sensitivities of 98.1% for the detection of RMP resistance. The sensitivities of the assays for the detection of INH resistance differed: 87.8% for the MTBDR assay and 90.2% for the MTBDRplus assay. The specificities of both assays were 97.8% for the detection of RMP resistance and 100% for the detection of INH resistance.

DISCUSSION

The results of the present study have shown that the MTB DRplus assay is easy to perform and has the capability for the rapid detection of RMP- and INH-resistant *M. tuberculosis*. As previously shown for other DNA strip assays (1, 11, 16, 17, 25, 29), the MTBDRplus assay has been proven to be suitable for application both with culture isolates and directly with smear-positive specimens.

With respect to culture isolates, the sensitivities of the MTBDR assay for the detection of RMP resistance were recently reported to be in the range of 95% to 99% (4, 10, 17). This is in concordance with the high sensitivity of the MTB DRplus assay measured in our study (98.7%). Both the rarity of RMP-resistance-associated mutations in codons other than the *rpoB* 81-bp hot spot region and the rarity of silent mutations in the hot spot region are responsible for the high rate of detection of RMP resistance by investigation of this region (9, 10, 12; this study). In this study, the S531L mutation in *rpoB* was the most frequent (78 of 106 strains [73.6%]), followed by mutations in codon 526 (16 of 106 strains [15.1%]). This is comparable to the frequencies reported in other studies (17), although the distribution can also differ in some settings (2). Although the prevalence of false-resistant and false-susceptible results seems to be infrequent, some authors have discussed the relevance of mutations in codon 533 to RMP resistance (15). *M. tuberculosis* isolates with mutations in codon 533 showed RMP susceptibility (15, 21) or low-level or high-level resistance (27, 30). Our data support the suspicion that mutations in codon 533 are not associated with RMP resistance and that RMP resistance in isolates with codon 533 mutations may be due to other mutations. The detection of a codon 533 mutation by the MTBDR and MTBDRplus assays is due to the omission of the respective wild-type *rpoB* band (WT 5 and WT 8, respectively). The recommendation in these cases is either to control the result by DNA sequencing or confirm the result by the conventional DST.

Some authors discussed a main limitation of the MTBDR test system, which is the low sensitivity for the detection of INH resistance (16, 17). This was due to the fact that the test targets only the *katG* S315T mutation. By using the MTBDR plus assay, this problem is now at least partly solved by the addition of a second target for the detection of INH resistance, the regulatory region of the *inhA* gene. In our study we could show that this addition led to an improvement in the rate of detection of INH resistance of 3%, although it can be expected that in other settings this advantage may be higher. Concerning the distribution of mutations associated with INH resistance, a disequilibrium between mono-INH^r isolates compared to those with resistance to more drugs was identified (8, 10). INH resistance-associated mutations and, in particular, *katG* 315 mutations were less prevalent in mono-INH^r isolates than in the other group of isolates. Although the number of samples used in the present study is too small for a statistical analysis, this trend can be approved.

As already reported for the MTBDR assay, the new MTB DRplus assay can be applied directly to smear-positive specimens. With a turnaround time of approximately 6 h, these techniques save several weeks of time, which is required for primary isolation and conventional DST. However, these molecular tests should not be applied alone and therefore cannot totally replace culture methods for several reasons: (i) apart from RMP and INH susceptibility testing, culture is needed for all other drug susceptibility tests; (ii) RMP and INH susceptibility must be confirmed, since the possibility that a strain is resistant cannot be excluded for a strain with a wild-type pattern by the MTBDR assay; and (iii) in the case of a mixed infection with an MTBC strain and a nontuberculous mycobacterium, interpretation of the MTBDR assay results could be difficult (data not shown). Furthermore, it should not be used as a nucleic acid amplification technique for the direct detection of MTBC strains in primary specimens. Nevertheless, the new MTBDRplus assay is a major improvement among assays for the routine detection of RMP- and INH-resistant MTBC strains, since with this rapid and reliable tool, the therapeutic management of patients can be optimized. This is of special importance for MDR strains and highly infectious patients, since the prevention of transmission of resistant strains is one of the challenges of the present and future.

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